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A review of potential environmental risks associated with the use of pesticides to treat Atlantic salmon against infestations of sea lice in southwest New Brunswick, Canada

Dr. Les Burridge

Fisheries and Oceans Canada, St. Andrews Biological Station,
531 Brandy Cove Road, St. Andrews, NB, E5B 2L9

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Canada

Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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ABSTRACT

Recently, pesticide bath treatments have been used in southwest New Brunswick in order to control sea lice on farmed Atlantic salmon in open netpens. As part of the emergency registrations for anti-sea lice bath treatments, Health Canada's Pest Management Regulatory Agency was seeking additional information on the biological effects of pesticides on non-target organisms. This review analyzed three pesticides evaluated for their biological effects on non-target organisms were: Salmosan® (active ingredient: azamethiphos), AlphaMax® (active ingredient: deltamethrin), and Paramove® 50 (active ingredient: hydrogen peroxide); however, only Salmosan® and Paramove® 50 are currently being used. This review found that in lab studies, acute lethal toxicity varied with the pesticide, the non-target species, and the life stage. Azamethiphos (active ingredient in Salmosan®) is a neurotoxin, and the formulation is soluble in water and therefore unlikely to accumulate in sediment or bioaccumulate in tissue. In acute lethal toxicity tests, adult lobster and shrimp were the most susceptible species to azamethiphos (48-h LC_{50} for adult lobster: 1.39 µg azamethiphos L^{-1}; 96-h LC_{50} for Mysids: 0.52 µg azamethiphos L^{-1}), while adult lobsters repeatedly exposed to below prescribed treatment concentrations showed sublethal behavioural effects, such as affecting reproduction in females. Deltamethrin is a synthetic pyrethroid which interferes with nerve membrane function and is known to be highly toxic to crustaceans (96-h LC_{50}: for adult lobsters - 1.4 ng L^{-1}; stage III and IV lobster larvae - 3.7 – 4.9 ng L^{-1} and 28.2 ng L^{-1}, respectively; amphipods – between 1.7 and 8.0 ng L^{-1}). It has low solubility in water and due to its high lipophilicity and adsorption coefficients can persist in sediments. It is rapidly metabolized by fish and therefore unlikely to accumulate in tissues. The hydrogen peroxide in Paramove® 50 forms bubbles in the gut and haemolymph to paralyze muscles, which causes sea lice to float to the water surface. It is fully miscible in water, does not persist or bioaccumulate, and degrades to oxygen and water in about 7 days. There is little information on the toxicity of hydrogen peroxide to marine organisms. Bath treatments against sea lice have been inconsistent and effectiveness is dependent on water temperature. In summary, in laboratory lethal toxicity tests with active ingredients, lobsters were consistently more sensitive to therapeutants than Crangon and Mysid shrimps tested. The degree of toxicity was therapeutant specific with Paramove® 50 being the least toxic of the three formulations tested, while AlphaMax® was the most toxic.

Examen des risques environnementaux potentiels liés à l'utilisation de pesticides pour traiter le saumon de l'Atlantique contre les infestations de pou du poisson dans le sud-ouest du Nouveau-Brunswick, au Canada

RÉSUMÉ

Récemment, des bains thérapeutiques de pesticides ont été utilisés dans le sud-ouest du Nouveau-Brunswick pour lutter contre les infestations de pou du poisson sur le saumon de l'Atlantique élevé dans des filets en eau libre. Dans le cadre des homologations d'urgence des bains thérapeutiques contre le pou du poisson, l'Agence de réglementation de la lutte antiparasitaire (ARLA) souhaitait obtenir des données supplémentaires sur les effets biologiques des pesticides sur les organismes non ciblés. Pour cet examen, on a analysé les effets biologiques de trois pesticides sur des organismes non ciblés : Salmosan® (ingrédient actif : azaméthiphos), AlphaMax® (ingrédient actif : deltaméthrine) et Paramove® 50 (ingrédient actif : peroxyde d'hydrogène). Toutefois, on n'utilise actuellement que Salmosan® et Paramove® 50. L'examen a permis de constater que, dans les essais de laboratoire, la toxicité aiguë létale variait selon le pesticide, les espèces non ciblées et le stade biologique. L'azaméthiphos (ingrédient actif du Salmosan®) est une neurotoxine dont la formulation est soluble dans l'eau, ce qui rend improbable son accumulation dans les sédiments ou sa bioaccumulation dans les tissus. Dans les essais de toxicité aiguë létale, les homards et les crevettes adultes étaient les espèces les plus vulnérables à l'azaméthiphos (48 h CL_{50} pour le homard adulte : 1,39 µg d'azaméthiphos l^{-1}; 96 h CL_{50} pour les mysidacés : 0,52 µg d'azaméthiphos l^{-1}) tandis que les homards adultes exposés à des concentrations inférieures aux concentrations prescrites pour le traitement affichaient des effets comportementaux sublétaux affectant, par exemple, la reproduction pour les femelles. La deltaméthrine est un pyréthroïde synthétique qui nuit à la fonction de la membrane des fibres nerveuses et dont la toxicité pour les crustacés est bien connue (96 h CL_{50} pour les homards adultes : 1,4 ng l^{-1}; larves de homard de stade 3 et 4 : respectivement de 3,7 à 4,9 ng l^{-1} et 28,2 ng l^{-1}; amphipodes : de 1,7 à 8.0 ng l^{-1}). En raison de sa faible solubilité et de ses coefficients élevés d'adsorption et de lipophilie, la deltaméthrine peut demeurer dans les sédiments. Elle est métabolisée rapidement par les poissons et son accumulation dans les tissus est donc peu probable. Le peroxyde d'hydrogène contenu dans Paramove® 50 forme des bulles dans les intestins et l'hémolymphe, ce qui paralyse les muscles et fait flotter le pou du poisson sur la surface de l'eau. Il est complètement miscible dans l'eau, ne persiste pas dans l'environnement et ne se bioaccumule pas, et se décompose en oxygène et en eau en environ 7 jours. Il y a peu de données sur la toxicité du peroxyde d'hydrogène pour les organismes marins. Les bains thérapeutiques contre le pou du poisson ont eu des résultats variables et leur efficacité dépend de la température de l'eau. En résumé, dans les essais de toxicité létale des matières actives en laboratoire, les homards étaient invariablement plus vulnérables aux agents thérapeutiques que la crevette de sable et la mysis effilée. Le degré de toxicité dépendait de l'agent thérapeutique : parmi les trois formulations testées, Paramove® 50 était la moins toxique et AlphaMax® la plus toxique.

INTRODUCTION

Cultured Atlantic salmon are susceptible to infectious bacterial and viral diseases and to infestations by parasites, such as sea lice. Sea lice are ectoparasites of many species of wild fish and are a serious problem for salmon aquaculture industries (Roth et al., 1993; MacKinnon, 1997). The species that infest cultured Atlantic salmon are *Lepeophtheirus salmonis* and *Caligus elongatus*. Infestations result in skin erosion and sub-epidermal haemorrhage which, if left untreated, would result in significant fish losses, probably as a result of osmotic stress and other secondary infections (Wooten et al., 1982; Pike, 1989). The first severe epidemic of sea lice in Atlantic Canada occurred in 1994 (Hogans, 1995). Sea lice reproduce year round and the aim of successful sea lice control strategy must be to pre-empt an internal infestation cycle from becoming established on a farm by exerting a reliable control on juvenile and preadult stages, thus preventing the development to gravid females (Treasurer and Grant, 1997). Effective control of sea lice infestations requires good husbandry and effective anti-parasitic chemicals (Rae, 2000; Eithun, 2004).

The types of therapeutants available for use and the treatment protocols are tightly regulated in Canada and therapeutants can only be used under prescription from a licensed veterinarian. Health Canada regulates chemotherapeutants used in the aquaculture industry, which are considered either a drug or a pesticide depending on the use and method of application. If the product is applied topically or directly into water, it is considered a pesticide; however, if a product is delivered through medicated feed or by injection, it is considered a drug. In order for pesticide formulations to be registered for use in aquaculture they must be shown to be efficacious, i.e., it will kill the target organism, it must be shown to be safe for the fish, and it must be shown to have an acceptable risk to non-target organisms (Peter Delorme, Pest Management Regulatory Agency, personal communication).

There are provisions for Emergency Registrations (ER) and 'off-label' use of drugs and pesticides. Pesticides are the responsibility of the Pest Management Regulatory Agency (PMRA) of Health Canada and are registered under the authority of the *Pest Control Products Act* (PCPA). The PCPA requires the registrant to submit environmental data as part of the registration process. Most data submitted to the regulatory agencies are proprietary and, as such, are not available to the general public but may be obtained by researchers (with restrictions) from Health Canada.

Aquaculture, like all forms of intensive food production, may generate environmental costs. Chemicals used in the treatment of sea lice infestations are subsequently released to the aquatic environment and may impact other aquatic organisms and their habitat. This paper will review the chemical therapeutants available to control sea lice in Canada and assess their risks to the aquatic ecosystem, particularly in the Bay of Fundy. The review will be limited to three pesticides currently, or recently, applied in the Bay of Fundy in southwest New Brunswick, Salmosan® (active ingredient: azamethiphos), Paramove® 50 (active ingredient: hydrogen peroxide) and AlphaMax® (active ingredient: deltamethrin).

The author has relied heavily on summary papers prepared by Burridge (2003), Haya et al. (2005) and Burridge et al. (2010) and Burridge et al. (2010a).

SEA LICE BIOLOGY

The life cycle of the sea louse *Lepeophtheirus salmonis* is shown in Figure 1. Adult females of *L. salmonis* are 8 to 12 mm in length, while males are about half of this size. The sea lice on cultured fish tend to be a bit smaller than those on wild fish. Sea lice eggs hatch directly into the water from egg strings fastened to the genital segment of females. The larvae are free-swimming nauplii through one moult and then become infective copepodids. These are about 0.7 mm long and 0.3 mm wide, and it is this stage that can recognize and become attached to a host fish. It is, however, observed that adult sea lice can transfer from fish to fish. The dispersion of the nauplii is primarily passive as the larvae drift in the water, but the vertical movements of the larvae (copepodids are positively phototaxic) will also influence their position in a water column. In total, the sea lice pass through 10 stages, with one moult between each stage (Rae, 1979).

Sea lice development rates are dependent on the sea temperature. It takes a male 42 days, and a female 50 days, to develop from egg to adult at 10°C. The sea lice can, however, tolerate relatively a large range of temperatures and can hatch and develop at as low as 2°C (Boxaspen and Naess, 2000).

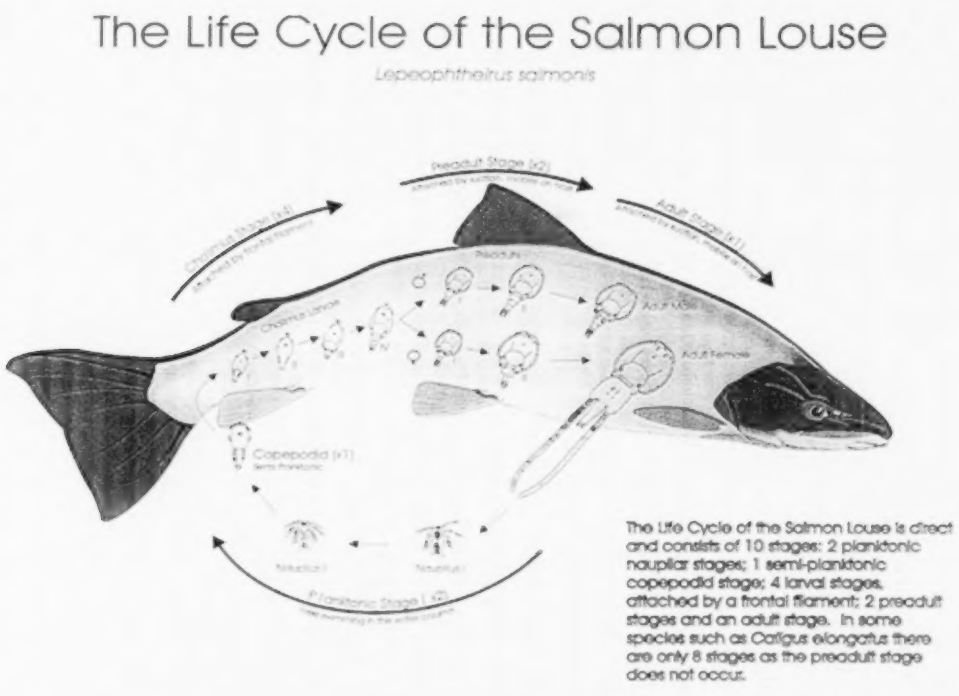
The Life Cycle of the Salmon Louse
Lepeophtheirus salmonis
Preadult Stage (x2)
Adult male (x1)
Chalimus Larvae
Adult female
Copepodid (x1)
Nauplius I
Nauplius II
Planktonic Stage I (x2)
The Life Cycle of the Salmon Louse is direct and consists of 10 stages: 2 planktonic naupliar stages; 1 semi-planktonic copepodid stage; 4 larval stages, attached by a frontal filament; 2 preadult stages and an adult stage. In some species such as Caligus elongatus there are only 8 stages as the preadult stage does not occur.

Figure 1. The life cycle of Lepeophtheirus salmonis (from Health Canada 2003).

The period during which a copepodid can infect a fish is called the infective window and is crucial in the control of sea lice. Larvae can infect fish from the first day after moulting, but they appear to be more infective after a few days. Longer than this and the copepodid exhausts its energy reserves, and becomes less successful in infecting susceptible host fish. Calculations on empirical data indicate that the latest day that a larva can infect fish is 32.5 days after

hatching at 6°C and 17 days at 12°C. Such long infective pelagic stages suggest that *L. salmonis* has a great potential for dispersion and that it can infect fish over a wide area away from the source. Thus, massive infection problems may be encountered by the salmon farming industry. This emphasizes the need for efficient husbandry strategies and chemical agents to control infections on fish farms and to reduce the potential for transfer of sea lice between farms. It also highlights the likelihood of this transfer in areas such as southwest New Brunswick where sites may be as close as 500 meters apart.

THERAPEUTANTS IN USE

As outlined, chemicals currently authorized for treating sea lice infestations are classified into two groups based on their route of administration, bath treatments or in-feed additives (Haya et al., 2005). This review will focus only on bath treatments.

In southwest New Brunswick bath treatments are conducted in one of three ways: skirting, tarping, and well boats. Skirt and tarp treatments involve reducing the depth of the net in the salmon cage, thus reducing the volume of water. The net-pen and enclosed salmon are either completely surrounded by an impervious tarpaulin (tarping) or a skirt is hung around the cage to a depth exceeding that of the enclosed salmon (skirting) and the chemical is added to meet the recommended treatment concentration. The salmon are maintained in the bath for a specified period (usually 30-60 minutes) and aeration/oxygenation may be provided. After treatment, the tarpaulin is removed and the treatment chemical is allowed to disperse into the surrounding water. Bath treatments are considered a topical application as the therapeutant is absorbed by the sea lice from the water.

Well boat treatments are conducted by pumping salmon into wells or treatment chambers on specially designed ships. Well boats used in southwest New Brunswick typically have two wells each capable of holding ~300-350 m^3 of water. Fish are pumped into these wells, allowed to acclimate for a short period of time and then pesticide is added to the appropriate concentration. Aeration/oxygenation may be provided. At the end of the prescribed treatment period (30-60 minutes) the wells are flushed by pumping in "clean" seawater. The fish are then pumped back into net pens.

The use of well boats generally leads to smaller quantities of pesticides being used in comparison with tarp or skirt bath treatments. A "typical" 100 m net pen, fully tarped at 3 m depth has a treatment volume of ~ 2250 m^3 while a skirt treatment of the same cage with a skirt depth of 4 m would result in a treatment volume of ~3000 m^3. Using a well boat the same net pen would require 4 wells for treatment, but with a maximum treatment volume of 1400 m^3. As such, well boats require only 46% of a skirted treatment and 62% of a full tarp treatment and a concomitant reduction in amount of pesticide used.

It is important to clearly distinguish between active ingredients and formulations. Active ingredients are the chemical compounds (pesticides) designed to kill the target organism (sea lice). The active ingredients are applied as part of pesticide formulations to optimize delivery, exposure and efficacy. The ingredients in the formulation will affect how the active compound behaves in the environment. While the PMRA requires data on the physical-chemical properties of the active ingredients as well as information on the constituents of the formulations, registration of a formulation is often completed without physical-chemical data specific to the formulation, solubility and partition co-efficients for example.

Pesticide formulations are prepared to optimise the probability that the active ingredient reaches and affects the target organism, in this case sea lice. The formulation, therefore, is prepared with a number of chemicals in addition to the active ingredient, which may include solvents, surfactants, and stabilisers. These chemical additions to pesticide formulations are proprietary and therefore not known to the researcher. In work conducted at our lab, formulations of the product used commercially were tested. Recommended treatment concentrations and chemical measurements are reported or prescribed on the basis of the active ingredient. In the absence of information on the constituents of the formulations, it is impossible to quantify their concentration and estimate thresholds.

Pesticide formulations have a defined therapeutic index defining the difference in effectiveness for killing sea lice and the level that will negatively affect salmon. Infestations of sea lice cause stress to salmon making them more susceptible to disease and further infestation by sea lice. Other treatment-related factors are also known to cause stress in salmon. Handling, crowding and short-term exposure to pesticide formulations may all result in a generalised stress response in salmon (Wendelaar Bonga, 1997). In a sea lice treatment context, these stressors are applied over short time periods (< 1 h) and stress responses are also of short duration. Fish are known to recover quickly to acute, short-term stressors (Wendelaar Bonga, 1997). In addition, developers and suppliers of anti-louse formulations report wide safety margins for their products and salmon, i.e., the recommended treatment concentration is well below thresholds of effects for salmon. Hydrogen peroxide is the lone exception, see Paramove® 50 section below.

This review focuses on three pesticide formulations that are either currently or have been recently used to control infestations of sea lice in New Brunswick. Each formulation has a different active ingredient and each active ingredient has a specific mode of action for killing sea lice. The three formulations are Salmosan®, AlphaMax®, and Paramove® 50 and the three active ingredients are azamethiphos, deltamethrin and hydrogen peroxide, respectively. All effects data presented in this review are reported as the concentration of the active ingredient.

Of the three pesticide formulations being considered for this review (Salmosan®, AlphaMax®, and Paramove® 50), only Paramove® 50 is fully registered for use in finfish aquaculture in Canada; however, its registration is for use in hatcheries and not for use as a bath treatment to control sea lice. Both hydrogen peroxide, as the product Salartect®, and azamethiphos, as the product Salmosan®, were previously registered as anti-louse treatments in Canada. Recently, both pesticides have been given emergency registration (ER) status and are being used to combat sea lice infestations in southwest New Brunswick (Kevin Wickens, Health Canada, PMRA, personal communication, 2012). Azamethiphos is still applied as Salmosan®; however, hydrogen peroxide is now applied as Paramove® 50. AlphaMax® was used under an ER from Health Canada in the fall of 2009 and the summer of 2010.

SALMOSAN®

Efficacy and Mechanism of Action of Azamethiphos

Azamethiphos is an organophosphate insecticide and the active ingredient in the formulation Salmosan®. The formulation is a wettable powder consisting of 47.5% azamethiphos. It is used as a bath treatment at 100 µg L^{-1} for 30-60 minutes in well boats and tarps and at 150 µg L^{-1} if applied as a skirt treatment. At water temperatures below 10°C, treatments can last up to 60 min at the discretion of the attending veterinarian. At water temperatures above 10°C, a 30 min treatment is recommended (Salmosan® product label). The product is effective only

against pre adult and adult sea lice and has no effect on the larval stages. This results in a need to treat cages repeatedly during periods of high infestation. The Pest Management Regulatory Agency's Emergency Registration for Salmosan® limits the application of Salmosan® to two treatments per day per aquaculture site. Azamethiphos has a therapeutic index for salmon of near 10 (Haya et al., 2005).

Azamethiphos has neuro-toxic action, acting as an acetylcholinesterase (AChE) inhibitor. In the absence of AChE activity, nerves repetitively fire and the affected organisms eventually die. Azamethiphos has been shown to be mutagenic in several *in vitro* tests (EMEA, 1999). DNA damage was induced in mammalian cell lines *in vitro* and azamethiphos induced an increase in revertant genes in the yeast *S. cerevisiae* D7, also *in vitro*. Zitko (2001) suggested that the high alkylating potency of azamethiphos could explain the mutagenic response and recommended that biological effects studies on non-target biota should include tests for delayed effects. However, *in vivo* studies with azamethiphos did not result in evidence of mutagenicity (EMEA, 1999). The reason for this could be related to experimental protocols or to metabolism of the product *in vivo*.

Sea lice sensitivity to azamethiphos is variable and some sea lice populations are more sensitive to this compound than others (Roth et al., 1996). Development of resistance to organophosphates is common and has been shown for azamethiphos (Levot and Hughes, 1989). In sensitive sea lice populations, azamethiphos is effective in removing >85 % of adult and pre-adult sea lice, but is not effective against earlier sea life stages of the parasite (Roth et al., 1996).

The use of Salmosan® was discontinued in Canada in 2002. The product had ceased to be effective, in-feed products were available and the registrant did not request a renewal of the registration through PMRA. Burridge et al. (2010) noted that after several years of no sales, Salmosan® was re-introduced as an anti-sea louse treatment in Europe in 2008. It was given an ER for use in New Brunswick in 2009.

Distribution and Fate of Azamethiphos

Azamethiphos is soluble in water (1.1 g L^{-1}) and has a low octanol-water partition coefficient ($\log K_{ow} = 1.05$) (SEPA, 2005). The $\log K_{ow}$ is the logarithm of the octanol-water partition coefficient. It is internationally accepted that $\log K_{ow} \geq 3$ indicates a potential to bioaccumulate and the Canadian Environmental Protection Act (CEPA) recognizes $\log K_{ow} \geq 5$ as indicative of potential to persist in the environment (Beek et al., 2000). Consequently, azamethiphos is likely to remain in the aqueous phase on entering the environment. It is unlikely to accumulate in tissue or in sediment. Azamethiphos decomposes by hydrolysis in natural water with a half-life of 8.9 days. Dispersion studies indicated that after release of an experimental treatment (200 µg L^{-1}), the concentration of azamethiphos was below detection (0.1 µg L^{-1}) in a short period of time. It was not detected below 10 m depth and it was suggested that it is unlikely that azamethiphos would accumulate in sediment (SEPA, 2005).

The bioaccumulation of azamethiphos by salmon is low and depletion of total azamethiphos in salmon is rapid and the pre-marketing withdrawal time is 24 h (EMEA, 1999).

Biological Effects of Salmosan® (Azamethiphos)

Laboratory Studies (published data)

Lobster and shrimp were the most susceptible species to azamethiphos in lab-based acute toxicity tests, while bivalves such as scallops and clams were unaffected (Burridge and Haya, 1998). The 48-h LC_{50}'s estimated for the first four larval stages and adults of the American lobster (*Homarus americanus*) after exposure to Salmosan® are: Stage I 3.57 μg L^{-1}, Stage II 1.03 μg L^{-1}, Stage III 2.29 μg L^{-1}, Stage IV 2.12 μg L^{-1}, and Adults 1.39 μg L^{-1} (Burridge et al., 1999). LC_{50}s are reported as the concentration of azamethiphos. There was no statistically significant difference among these values. There is a seasonal aspect to susceptibility of American lobsters to azamethiphos. Female lobsters are significantly more sensitive to azamethiphos in the summer than at any other time of year (Burridge et al., 2005). For Adult and Stage IV lobsters exposed repeatedly for varying lengths of time to 4 concentrations of azamethiphos (Burridge et al., 2000), the No Observed Effect Concentration (NOEC) was nine exposures of 30 min each over three days to 1 μg L^{-1} of azamethiphos. In addition to observed lethality, many surviving lobsters showed significant behavioural responses, after repeated exposure to concentrations of 10 μg L^{-1} (see description below).

Research commissioned by Ciba Geigy shows that azamethiphos is only lethal to several groups of invertebrates (bivalve molluscs and gastropods, amphipods, and echinoderms) at concentrations greater than the prescribed treatment concentration of 100 μg L^{-1} (SEPA, 2005). The 24-h LC_{50} of azamethiphos to the copepod, *Temora longicornis*, is reported to be >10 μg L^{-1}. The 96-h LC_{50} for European lobster larvae, *Homarus gammarus*, is 0.5 μg L^{-1} and is in general agreement with the 48-h LC_{50} for the American lobster, 1.39 μg L^{-1} (Burridge et al., 1999). Finally, the 96-h LC_{50} for the mysid shrimp, *Mysidopsis bahia*, is reported as 0.52 μg L^{-1} (SEPA, 2005).

In laboratory studies, American lobsters exposed to Salmosan® (5.0-10.0 μg (azamethiphos) L^{-1}) became quite agitated, often 'flopping' erratically around the exposure tank (Burridge et al., 2000). They were also aggressive to other lobsters and reacted very quickly to any movement. They seemed to lose control of their claws and eventually flipped onto their backs and died within hours. Some affected lobsters remained moribund for periods of time ranging from hours to days. The consequences of behavioural responses such as these on organisms and populations in the natural environment are unknown.

Laboratory studies were conducted to investigate possible sublethal effects of Salmosan® exposure on American lobster. Preovigerous females were exposed for 1 h biweekly to 10 μg L^{-1} azamethiphos and monitored for spawning success and survival (Burridge et al., 2008). Surprisingly, even with such infrequent exposures, up to 100% of the animals exposed to this concentration died during the experiment: some expired after only three treatments. At lower concentrations a significant number of the surviving lobsters failed to spawn. A laboratory study indicated that shelter use behaviour could be affected by Salmosan® (Abgrall et al., 2000). However, exposure to concentrations of azamethiphos in water was greater than five times the recommended treatment concentration for periods of several hours.

Ernst et al. (2001) measured the toxicity of Salmosan®, as azamethiphos, to a number of species including: the bacterium (*Vibrio fisheri*); the Green sea urchin (*Strongylocentrus droebrachiensus*), the Painted sea urchin (*Lytechinus pictus*) (fertilization); the Threespine stickleback (*Gasterosteus acualeatus*); three amphipods (*Amphiporeia virginiana*, *Gammarus spp*; and *Eohaustorius estuaries*); a polychaete (Polydora cornuta); Brine shrimp (*Artemia salina*); and a rotifer

(*Brachionus plicatilis*). They determined that amphipods were most sensitive with *Eohausorius estuarius* having a 48h EC50 (immobilization) of approximately 3 µg L^{-1}.

The response of mussels to stimuli was unaffected by exposures to 10.0 µg L^{-1} for up to 24 h (SEPA, 2005). The inhibition of AChE by azamethiphos is not cumulative in fish (Roth et al., 1993). However, cumulative inhibition of AChE occurred in lobster in studies to determine the effect of Salmosan® on spawning (Burridge et al., 2008). Mussel closure rate was affected at concentrations above 100 µg L^{-1} and exposure to 46.0 µg L^{-1} resulted in 50% inhibition of AChE activity (SEPA, 2005). AChE activity in herring yolk sac larvae and post-yolk sac larvae was inhibited by 96-h exposure to azamethiphos at 33.4 and 26.6 µg L^{-1}, respectively. Herring larvae were reported to tolerate azamethiphos better than another organophosphate, DDVP (Roth et al., 1993).

Biological effects of Salmosan® (unpublished results)

In 2011 staff at the St. Andrews Biological Station conducted a series of bioassays to determine the acute response of several invertebrate species to Salmosan® (Table 1). Preliminary results show that no LC_{50} could be determined for Stage I lobster larvae, the mysid shrimp, *Mysis stenolepsis* or the sand shrimp, *Crangon septemspinosa* after a 1-h exposure to 100 µg azamethiphos L^{-1} followed by 95-h in clean water. The LC_{50} for adult lobsters was estimated to be 24.8 µg azamethiphos L^{-1}. Table 1 also includes estimates of the No Observable Effect Concentrations (NOEC) based on lethality and dilution factors to reach these thresholds based on the recommended treatment concentration (100 µg azamethiphos L^{-1}). Table 2 shows the LT_{50} estimates for the two concentrations of azamethiphos that resulted in >50% mortality of exposed organisms following 1-h exposures and 95-h of monitoring. Under these conditions only adult lobsters were killed with >50% mortality occurring very quickly or not at all. When adult lobsters were exposed to Salmosan® continuously for 10 days the LC_{50} was estimated to be 0.216 µg azamethiphos L^{-1} (Table 6.).

Table 1. The LC_{50} and estimated NOEC of Salmosan® expressed as the measured concentration of azamethiphos, to mysids, Crangon, adult and Stage I of the American lobster. Dilution factors are based on a recommended treatment concentration of 100 µg L^{-1} (as azamethiphos) as this is the concentration applied in southwest New Brunswick. Test organisms are exposed for 1 hour and held for a further 95 h. Water temperature was 10.8°C.

Species/Life Stage	LC_{50} (95% C.I) µg L^{-1}	Dilution factor	NOEC (lethality)	Dilution factor
Lobster I	>86.5	<1.15	<0.37	>270
Lobster Adults	24.8 (21.7-27.9)	4.0	9.85	10.2
Mysids	>85.5	<1.17	<0.97	>100
Crangon	>85.5	<1.17	<0.97	>100

Table 2. LT_{50} (h) of Salmosan® to various invertebrate species from southwest NB. Results are from 1-h exposure and 95-h post-exposure monitoring. Water concentrations are expressed as the measured concentration of azamethiphos.

Concentration µg L^{-1}	Stage I Lobster	Adult Lobster	Mysids	Crangon
85.5	>95	0.75	>95	>95
27.7	>95	2.5	>95	>95

Field Studies with Salmosan®

During 1995, a study was conducted to determine the effects of single operational Salmosan® treatments on juvenile and adult American lobsters, shrimp *(Pandalus montagui)*, clams (*Mya arenaria*), and scallops (*Placopecten magellanicus*), suspended at two depth and varying distances from the treated cage. During two of the treatments, all lobsters held within the treatment tarpaulin died (Chang and McClelland, 1996). No other treatment-related mortalities were observed. In addition, no mortalities were observed with lobsters that were suspended at three depths at 20 sites surrounding a salmon cage site that was conducting operational treatments with Salmosan®. Mussels deployed during field trials in Scotland were unaffected (SEPA, 2005). Mortality among lobster larvae was 27% but was not correlated to distance from the treatment cage.

The amphipod, *Eohaustorius estuarius,* was used as test organism in a dye dispersion study designed to simulate net-pen releases. The study used a rhodamine dye as a tracer and found that 1/200 - 1/3000 the release concentration were not achieved until post-release times ranging from 2-5.5 h. Most samples from the plume were not toxic when azamethiphos was the test pesticide and none were toxic past 20 minute post release. Ernst et al. (2001) suggest that Salmosan® presents a lower environmental risk than the other pesticide they tested during that study, cypermethrin.

Finally, survival of American lobsters suspended at mid-depth and near bottom at four sites in the salmon farming area of Lime Kiln Bay, New Brunswick, Canada, plus a control site, was monitored for nine weeks during August-October 1996. There were no apparent differences in lobster survival between the experimental and control sites (Chang and McClelland, 1997). No residues of azamethiphos were detected in water samples collected weekly from the five sites (Detection Limit = 50 pg L^{-1}). Diving surveys at a lobster nursery area located near a salmon farm in early August, September and late October of 1996 found no apparent changes in lobster populations over time, and the area was found to have a considerable population of juvenile lobsters.

Measurements of primary productivity and dissolved oxygen were made before, during and after chemical treatments at salmon farms in southwest New Brunswick in August-September 1996. There were no evident effects on dissolved oxygen and chlorophyll *a* levels, indicating no impact on primary production (David Wildish, St. Andrews Biological Station, St. Andrews, NB, unpublished data).

ALPHAMAX®

Efficacy and Mechanism of Action of Deltamethrin

Pyrethrins are the active constituents of an extract from flower heads of *Chrysanthemum cinerariaefolium*. This mixture of chemically related compounds has been used for their insecticidal activity since the late 19th century (Davies, 1985). The pyrethrins decompose readily as they are susceptible to catabolic enzymes and sunlight. In the early 1960s, synthetic analogues that were more persistent than the natural pyrethrins were developed and referred to as pyrethroids (Barthel, 1961). It was their high degradability, low toxicity to mammals and high toxicity to crustaceans that led to the initial interest in pyrethins and pyrethroids as treatments for sea lice infestations.

Deltamethrin is the active ingredient in the formulation AlphaMax®. Deltamethrin makes up 1% of the formulation, the remaining solvents, surfactants and other formulation products are not publically known. AlphaMax® was given an ER for use in southwest New Brunswick in 2009 and 2010.

The mechanism of action of the pyrethroids involves interference with nerve membrane function, primarily by their interaction with sodium (Na^+) channels (Miller and Adams, 1982) which results in depolarization of the nerve ending. In the case of the synthetic pyrethroid deltamethrin, this interaction results in repetitive firing of the nerve ending resulting in eventual paralysis and death (Crane et al., 2011).

The recommended treatment of salmon against sea lice is a 40 minute bath with AlphaMax® with a target concentration of 2.0 µg deltamethrin L^{-1} in tarped cages (SEPA, 2005). Deltamethrin is effective against all attached stages including adults, and therefore less frequent treatments should be required than with organophosphates; 5-6 week intervals rather then 2-3 week intervals, respectively (Haya et al., 2005).

In one of five Norwegian salmon sites that used deltamethrin for the treatment of sea lice, there was a significant decrease in effectiveness of the treatment with an increase in the number of treatments (Sevatadal and Horsberg, 2003). Bioassays using pre-adult stage II sea lice under laboratory conditions verified that resistance contributed to treatment failure, and that the EC_{50} was 25 times higher than at an area previously unexposed.

Distribution and Fate of Deltamethrin

Synthetic pyrethroids are unlikely to accumulate to a significant degree in fish and aquatic food chains since they are rapidly metabolized (Kahn, 1983). Deltamethrin has a very low water solubility (<2 µg L^{-1}) and a K_{ow} of 4.6 (Tomlin, 1994) indicating that deltamethrin can persist in sediments for weeks and may be desorbed and affect benthic invertebrates (Haya et al., 2005). Much of the available information on deltamethrin comes from the freshwater literature although several recent publications have addressed its use in marine waters (Gross et al., 2008; Fairchild et al., 2010; Crane et al., 2011).

Deltamethrin's high toxicity and rapidity of action could cause significant harm to limnic ecosystems after direct treatment (Thybaud, 1990). The adsorption of pyrethroids onto suspended solids can produce dramatic reductions in the apparent toxicity of the compound. The 96-h LC_{50} value for rainbow trout is 0.1-0.5 µg L^{-1} (NRCC, 1986). When trout were caged in a pond containing 14-22 mg L^{-1} suspended solids, the 96-h LC_{50} was 2.5 µg L^{-1}. In a pond

sprayed with deltamethrin containing 11 and 23 mg L^{-1} suspended solids, deltamethrin partitioned rapidly to suspended solids, plants, sediment and air with a half-life of 2-4 h in water (Muir et al., 1985). Because pyrethroids tend to adsorb onto particulate matter, chronic exposures may not occur other than in laboratory studies.

Biological Effects of AlphaMax® and Deltamethrin (published)

The impact of pyrethroids on non-target aquatic animals, especially invertebrates has been reviewed (Mian and Mulla, 1992). In general pyrethroids are more toxic to non-target insects and crustaceans than to other phylogenetically distant invertebrates. Among arthropods, however, crustaceans are phylogenetically closer to insects than molluscs and showed noticeable sensitivity (Hill, 1985; Haya et al., 2005).

Deltamethrin is extremely toxic to crustaceans. The 96-h LC_{50} for adult lobsters was determined to be 0.0014 µg L^{-1} (1.4 ng L^{-1}) for deltamethrin in the agricultural formulation Decis® (Zitko et al., 1979). Fairchild et al. (2010) reported the 96-h LC_{50} for deltamethrin in the AlphaMax® formulation was 3.7 – 4.9 ng L^{-1} for Stage III lobster larvae and 28.2 ng L^{-1} for Stage IV post-larvae. The 96-h LC_{50} for the amphipod, *Eohaustorius estuarius*, was between 1.7 and 8.0 ng L^{-1}. The sand shrimp was less sensitive to AlphaMax® with a 96-h LC_{50} of 45.3 ng L^{-1} (Fairchild et al., 2010). These authors exposed these invertebrates to various formulations of deltamethrin and for various lengths of time including 1-h exposures followed by 95 h or 16 days in clean water. The LC_{50}s determined after only 1-h exposure were 36.5, 13.3 and 142 ng L^{-1} for Stage III lobster larvae, *E. estuarius* and *C. Septemspinosa*, respectively. Recognizing that the reported toxicity values may overestimate risk potentials because of their higher exposure periods than would be expected after operational treatments, the data of Zitko et al. (1979) were re-calculated by Ernst et al. (2001) to derive lethal concentrations for short term exposures. Those calculations indicated that approximately 0.5 µg L^{-1} would be toxic to adult lobsters for exposure periods of about 6 h, which correlate with the dispersion measurements of Ernst et al. (2001). Gross et al. (2008) also report Brown shrimp toxicity of 0.14 µg L^{-1} for 6-h exposures. Those values represent a dilution of 1/10 - 1/35 of the recommended treatment concentration. According to the data from Ernst et al. (2001), these dilutions could occur from 5 minutes to 1 hour post-release.

Because of its lack of water solubility, high lipophilicity and high adsorption coefficients, deltamethrin is predicted to absorb preferentially to particles, particularly those with high organic content and to sequester to bottom sediments (Muir et al., 1985). The half-life for deltamethrin in marine sediments has been estimated at approximately 140 days, indicating that multiple treatments may result in accumulation of this compound in sediments near cage sites (Gross et al., 2008).

There are some data which suggest that deltamethrin may have a sublethal effect on the immune function of fish (Pimpão et al., 2007; 2008); however, the exposure to pesticide was by injection and the environmental relevance is unclear.

Biological effects of AlphaMax® (unpublished results)

In 2010 and 2011 staff at the St. Andrews Biological Station conducted a series of bioassays to determine the acute response of several invertebrate species to AlphaMax®. These data are summarised in Tables 3 and 4. Briefly, estimates of the 24-h LC_{50}s for Stage I, II and IV lobster larvae are 0.8, 0.6 and 1.7 ng L^{-1}, respectively. These estimates are based on average measured concentrations of deltamethrin in exposure tanks. The 24-h LC_{50} for adult lobsters

was estimated to be 15 ng L^{-1}, considerably lower than the 6 h estimate of 0.5 µg L^{-1} (500 ng L^{-1}) derived by Ernst et al. (2001) using data from Zitko (1979). The mysid shrimp, *M. stenolepsis*, was equally sensitive to AlphaMax® as lobster with a 24-h LC_{50} of 1.4 ng deltamethrin L^{-1}. *C. septemspinosa* was less sensitive with a 24-h LC_{50} of 27 ng deltamethrin L^{-1}). Table 3 also includes estimates of the NOEC based on lethality and dilution factors to reach these thresholds based on the recommended treatment concentration (2 µg deltamethrin L^{-1}).

Recently the same species were exposed to AlphaMax® for 1 h followed by holding for 95 h in clean seawater (Table 4). The LC_{50} estimates based on measured concentrations of deltamethrin are as follows: Stage I lobster larvae 3.4 ng L^{-1}, adult lobsters 18.8 ng L^{-1}, *M. stenolepsis* 13.9 ng L^{-1}. Table 4 also includes estimates of the NOEC and dilution factors based on prescribed treatment concentrations.

Table 5 shows the LT_{50} estimates for a number of concentrations of deltamethrin found to result in >50% mortality of exposed organisms during 1-h exposures followed by 95-h of monitoring. High concentrations of deltamethrin are necessary to kill >50% of exposed Crangon but this threshold is met quite quickly (<5 h). Interestingly, and possibly of concern, is the observation that a 1-h exposure of Stage I lobster larvae to AlphaMax® can result in >50% mortality of exposed animals several days later (Table 5). A 10-day constant exposure of adult lobsters to AlphaMax® resulted in an LC_{50} estimate of 14.7 ng L^{-1} based on measured concentrations of deltamethrin (Table 6).

Table 3. The 24-h LC_{50} and estimated NOEC of AlphaMax®, expressed as deltamethrin, to mysids, Crangon and various life stages of the American lobster. Dilution factors are based on prescribed treatment concentrations of 2000 ng L^{-1} as deltamethrin. Results are based on measured concentrations of deltamethrin. Water temperatures ranged from 11.5 to 13°C.

Species/Life Stage	LC_{50} (95% C.I.) ng L^{-1}	Dilution factor	NOEC (lethal)	Dilution factor	NOEC (sublethal)	Dilution factor
Lobster I	0.8 (0.6-1.0)	2500	<0.08	25000	<0.08	25000
Lobster II	0.6 (0.3-1.0)	3300	<0.08	25000	<0.08	25000
Lobster IV	1.7 (0-4.8)	1200	<0.08	25000	<0.08	25000
Lobster Adults	15 (11-19)	130	4.8	420	<0.6	3300
Mysids	1.4 (0-3.6)	1400	<0.2	10000	<0.2	10000
Crangon	27 (14-40)	75	5	400	<8	250

Table 4. Estimates of LC_{50} and NOEC of AlphaMax®, expressed as the measured concentration of deltamethrin, to mysids, Crangon and Stage I and adult American lobster. Dilution factors are based on a recommended treatment concentration of 2000 ng L^{-1} as deltamethrin. Test organisms are exposed for 1 hour and held for a further 95 h. Water temperatures ranged from 8.5 (adult lobsters) to 13.4°C (mysids).

Species/Life Stage	LC_{50} (95% C.I.) ng L^{-1}	Dilution factor	NOEC (lethality)	Dilution factor
Lobster I	3.4 (1.5-6.0)	588	<0.6	3300
Lobster III	36.5 (25.0-53.3)*	55		
Lobster Adults	18.8 (3.90-33.6)	106	3.6	555
Mysids	13.9 (10.9-17.7)	51	0.9	2222
Crangon	142 (104-194)**	14		

*1-h exposure followed by 16 days in "clean" water from Fairchild et al. (2010)

** From Fairchild et al. (2010) at 15-16°C

Table 5. LT_{50} (h) of AlphaMax® to various invertebrate species from southwest NB. Results are from 1-h exposure and 95-h post exposure monitoring. Water concentrations are ranges of measured concentrations of deltamethrin from several bioassays except for data on Stage III lobster larva and Crangon which are nominal concentrations from Fairchild et al. (2010).

Concentration ng L^{-1}	Stage I	Stage III*	Adults	Mysids	Crangon*
1000	ND	4.9	ND	ND	4.9
320	ND	ND	ND	ND	4.9
75-148	50	ND	5.5	20	ND
22-48	55	ND	5	>95	ND
7.6-8.3	42	ND	>95	>95	ND
2.5-6.7	37	>384	>95	>95	ND
1.0-2.1	42	>384	>95	>95	ND

* From Fairchild et al. (2010)

ND = not determined

Table 6. The 10-day LC_{50} and estimated NOEC of AlphaMax® and Salmosan® to adult lobsters (expressed as measured deltamethrin and azamethiphos, respectively). Dilution factors are based on recommended treatment concentrations of 2 µg L^{-1} and 100 µg L^{-1} for deltamethrin and azamethiphos, respectively. Test organisms are exposed continuously for 10 days. Water temperature ranged from 11-14°C.

Compound	LC_{50} (95% C.I.) ng L^{-1}	Dilution factor	NOEC (ng L^{-1})	Dilution factor
Deltamethrin	14.7 (7.70-21.6)	136	5	400
Azamethiphos	216 (157-273)	463	125	800

PARAMOVE® 50

Efficacy and Mechanism of Action of Hydrogen Peroxide

Hydrogen peroxide is a strong oxidizing agent that was first considered for the treatment of ecto-parasites of aquarium fish (Mitchell and Collins, 1997). It is widely used for the treatment of fungal infections of fish and their eggs in hatcheries (Rach et al., 2000) and is registered in Canada by PMRA for that purpose. With the development of resistance of sea lice to organophosphates, it was preferable to use of hydrogen peroxide to treat infestations of both *L. salmonis* and *Caligus elongatus* (Jones et al., 1992). Hydrogen peroxide was used in salmon farms in Faroe Islands, Norway, Scotland and Canada in the 1990's (Treasurer and Grant, 1997). Hydrogen peroxide (Paramove® 50, Salartect®) is still authorized for use in Canada but its specific use as an anti-sea louse pesticide requires an ER. From 2000 - 2010 it was not used, or used sparingly, for the treatment of sea lice infestations in Canada.

The suggested mechanisms of action of hydrogen peroxide are mechanical paralysis, peroxidation by hydroxyl radicals of lipid and cellular organelle membranes, and inactivation of enzymes and DNA replication (Cotran et al., 1989). Most evidence supports the induction of mechanical paralysis when bubbles form in the gut and haemolymph and cause the sea lice to release and float to the surface (Bruno and Raynard, 1994).

Hydrogen peroxide has a half-life in seawater of about 7 days and it degrades to oxygen and water (Haya et al., 2005). Hydrogen peroxide is perceived as being of relatively low risk as a sea lice treatment; however, there is very little information on the non-target effects of the use of this chemical. It is known to have toxic effects to Atlantic salmon at concentrations of 2.4 g L^{-1}, which is near the treatment concentrations of 0.5 g L^{-1} (Haya et al., 2005).

The recommended dosage for bath treatments is 1.2 g L^{-1} for 40 min but the effectiveness is temperature dependent and Treasurer et al. (2000) suggest the compound is not effective below 10°C. In southwest New Brunswick treatments are the norm at these temperatures and use of hydrogen peroxide is monitored carefully at temperatures above 10°C due to a low therapeutic index for salmon and hydrogen peroxide is not recommended as a treatment for sea lice infestations at water temperatures above 14°C (Dr. Michael Beattie, Province of New Brunswick, personal communication). Treasurer et al. (2000) also state that treatments are rarely fully effective, but 85-100% of mobile stages may be removed. Hydrogen peroxide has little efficacy against larval sea lice and its effectiveness against pre-adult and adult stages has been inconsistent (Mitchell and Collins, 1992). Effectiveness can be difficult to determine on

farms as the treatment concentration varies due to highly variable volumes of water enclosed in the tarpaulin. Temperature and duration also influence the efficacy. Ovigerous females are less sensitive than other mobile stages (Treasurer et al., 2000). It is possible that a proportion of the eggs on gravid female sea lice may not be viable after exposure to hydrogen peroxide (Johnson et al., 1993). Hydrogen peroxide was less efficacious when treating sea lice infestations on salmon in a cage that had been treated regularly for 6 years than in cages where the sea lice were treated for the first time. This suggested that *L. salmonis* had developed some resistance to hydrogen peroxide (Treasurer et al., 2000).

In a laboratory experiment, all adult and pre-adult sea lice exposed to 2.0 g L^{-1} hydrogen peroxide for 20 min became immobilized, but half had recovered 2 h post-treatment (Bruno and Raynard, 1994). The recovered sea lice swam normally and may have been able to reattach to the host salmon (Hodneland et al., 1993). Therefore it was recommended that floating sea lice should be removed. However, re-infection has not been noticed in practice (Treasurer et al., 2000) as the removed sea lice generally show little swimming activity. Re-infection in the field is less likely because the free sea lice will be washed away with the tidal flow or eaten by predators. After treatment of a cage with approximately 1.5 g L^{-1} hydrogen peroxide at 6.5°C, all the sea lice that were collected from surface water of treated cages were inactive, but recovery commenced within 30 minutes and 90-97% of the sea lice were active 12 h post-treatment (Treasurer and Grant, 1997). In this study, a higher proportion of pre-adult sea lice was removed than of adult sea lice.

Distribution and Fate of Hydrogen Peroxide

Hydrogen peroxide is fully miscible in water and has a calculated K_{ow} of less than 1 (K_{ow} =-1.5) indicating no potential for persistence or bioaccumulation (HERA project, 2005). Hydrogen peroxide is generally considered to be the treatment method of lowest environmental risk because it decomposes into oxygen and water. At 4°C and 15°C, 21% and 54%, respectively, of the hydrogen peroxide has decomposed after 7 days in sea water. If the sea water is aerated the amount decomposed after 7 days is 45% and 67%, respectively (Bruno and Raynard, 1994). Field observations suggest that decomposition in the field is more rapid, possibly due to reaction with organic matter in the water column, or decomposition catalyzed by other substances in the water, such as metals.

Biological Effects of Hydrogen Peroxide (published)

There is little information on the toxicity of hydrogen peroxide to marine organisms. Most toxicity data are related to the potential effects on salmonids during treatment of sea lice infestations. Experimental exposure of Atlantic salmon to hydrogen peroxide at varying temperatures shows that there is a very narrow margin between treatment concentration (0.5 g L^{-1}) and that which causes gill damage and mortality (2.38 g L^{-1}) (Keimer and Black, 1997). As can be expected, hydrogen peroxide is toxic to crustaceans with a 24-h LC_{50} to the Brine shrimp (*Artemia salina)* of 0.8 g L^{-1} (Mathews, 1995). Hydrogen peroxide has been shown to cause a decrease in aerobic metabolic rate and intracellular pH in the sand shrimp (*Crangon crangon)* at concentrations of 0.68 g L^{-1} as a result of 5-h exposures (Abele-Oeschger et al., 1997). Those concentrations are one-half to two-thirds of the prescribed treatment concentration (1200 ppm).

Toxicity to fish varies with temperature; for example, the 1-h LC_{50} to Rainbow trout at 7°C was 2.38 g L^{-1}, at 22°C was 0.218 g L^{-1} (Mitchell and Collins, 1997) and for Atlantic salmon increased fivefold when the temperature was raised from 6°C to 14°C (Roth et al., 1993). There

was 35% mortality in Atlantic salmon exposed to hydrogen peroxide at 13.5°C for 20 min. Bruno and Raynard (1994) reported that there was a rapid increase in respiration and loss of balance, but if the exposure was at 10°C there was no effect. There is evidence that the concentrations of hydrogen peroxide used in sea lice treatments can cause gill damage and reduced growth rates for two weeks post treatment (Carvajal et al., 2000).

Abele-Oeschger et al. (1997) reported that hydrogen peroxide can affect the metabolism of the shrimp *C. crangon*. These authors were discussing peroxide in episodic rainfall with relatively low concentrations (micro-molar). However, this could be representative of diluted effluent from a cage treatment. None of the authors referred to above state whether or not the hydrogen peroxide used was in a formulation licensed for aquaculture use.

Biological Effects of Hydrogen Peroxide (unpublished results)

In 2011 staff at the St. Andrews Biological Station conducted a series of bioassays to determine the acute response of several invertebrate species to Paramove® 50 (Table 7). As expected this product is much less lethal to the aquatic invertebrates tested than Salmosan® or AlphaMax®. When experimental animals were exposed to Paramove® for 1 h then monitored for a further 95 hours, the LC_{50} estimate for Stage I lobster larvae was 1637 ppm, while adult lobsters survived exposure to 3750 mg L^{-1}, approximately three times the prescribed treatment concentration. The LC_{50} for Paramove® 50 and *M. stenoplepsis* was estimated to be 973 mg L^{-1}. The LC_{50} for *C. septemspinosa* was estimated to be 3182 mg L^{-1}. Table 7 also includes estimates of the NOEC and dilution factors based on prescribed treatment concentrations (1200 mg L^{-1}).

Table 8 shows estimates of the LT_{50} for several concentrations of hydrogen peroxide. The estimates were made from data collected during 1-h exposures followed by 95 h of monitoring. The table shows that death occurs quickly at or above the recommended treatment concentration especially with adult lobsters and mysids. At 950 mg L^{-1} mysids are the only species where >50% of exposed animals die, which took > 80 hours for this to occur. The 50% lethal threshold was not met for other species exposed to this concentration.

Table 7. The LC_{50} and estimated NOEC of Paramove® 50 expressed as hydrogen peroxide, to mysids, Crangon, adult and Stage I of the American lobster. Recommended treatment concentration is 1200 mg L^{-1} as hydrogen peroxide. Results are based on measured concentrations. Test organisms are exposed for 1 hour and held for a further 95 h. Water temperatures ranged from 9-10°C.

Species/Life Stage	LC_{50} (95% C.I.) mg L^{-1}	Dilution factor	NOEC (lethality)	Dilution factor
Lobster I	1637 (1385-2004)	ND*	356	3.4
Lobster Adults	>3750	ND	971	1.2
Mysids	973 (668-1427)	1.2	<245	5.0
Crangon	3182 (2539-5368)	ND	<223	5.4

*ND – Not determined

Table 8. LT_{50} (h) of Paramove® 50 to various invertebrate species from southwest NB. Results are from 1-h exposure and 95-h post-exposure monitoring. Water concentrations are expressed as measured concentrations of hydrogen peroxide.

Concentration mg L^{-1}	Stage I Lobster	Adult Lobster	Mysids	Crangon
3700	12	>95	0.3	1.4
1800	>95	2.5	1.4	>95
950	>95	>95	83	>95

DISCUSSION

The terms of reference for this paper ask a simple question: What are the known biological effects of hydrogen peroxide, azamethiphos and deltamethrin on key non-target organisms? The author believes it is also of use to briefly discuss the relative toxicities of these compounds.

Table 9 lists the US Fish and Wildlife Services rating system for acute toxicity of chemicals in aquatic systems. Using this rating system and our most sensitive species, both deltamethrin and azamethiphos can be considered super toxic. Hydrogen peroxide would be considered practically non-toxic. Despite the super toxic rating for both azamethiphos and deltamethrin, the latter is up to 5 orders of magnitude more lethal than the former using data for Stage I lobster larvae.

Data presented in Table 3 shows the lethality of AlphaMax® to a number of indigenous species. It should be noted that some of the data were provided by collaborators from Fisheries and Oceans Canada (DFO) and Environment Canada labs in Moncton. Their experiments were conducted at temperatures a few degrees warmer than exposure temperatures at St. Andrews Biological Station. Pyrethroids have been shown to be more toxic at lower temperatures (Sparks et al., 1983). It is unlikely that the temperature difference will affect relative lethality in general; however, it may affect comparison of absolute LC_{50} estimates. It is also noteworthy that the estimated LC_{50} for AlphaMax® to adult lobsters was not significantly different between 24-h and 10-day exposures. There was a difference between 1-h and 24-h exposures with adults however. For Stage I larvae, the LC_{50} estimates for AlphaMax® are the same for a 1-h exposure as for a 24-h exposure indicating that, at least for this life stage, a brief exposure results in the same consequence. Fairchild et al. (2010) have shown a similar result with Stage III lobster larvae. A 1-h exposure with this stage followed by 16 days post treatment monitoring provided the same LC_{50} estimate for 24 h and 16 days.

AlphaMax® is also an order of magnitude more lethal than another pyrethroid-based anti-sea louse formulation, Excis® (a.i. cypermethrin), currently used in Europe and the UK. In 24-h studies with adult lobsters, the LC_{50} for deltamethrin is reported as 15 ng deltamethrin L^{-1} compared to 140 ng cypermethrin L^{-1}. In a recently published paper, Palmquist et al. (2011) suggest that use of very sensitive organisms, in their case *Hyalella azteca*, should be discouraged when assessing the risk of deltamethrin. While they correctly suggest that lab-based studies may not fully reflect routes of exposure in the field, ignoring or downplaying data indicating that any product is lethal in the ng L^{-1} range seems ill-advised. The argument may be moot with respect to the data presented herein as treatment concentrations appear to be

environmentally relevant and the species tested are not only indigenous but, as with lobsters, are also commercially important.

Crane et al. (2011) suggest environmental concentrations as low as 1.4 ng L^{-1} should be sufficiently protective for sensitive saltwater species exposed to AlphaMax® for 48 h. These authors based their assessment on LC_{10} values calculated using toxicity data from a number of studies and a number of species. Data presented here, see Table 10 for example, clearly show that concentrations of that magnitude for that time period would be lethal to lobster larvae and likely lethal to mysid shrimp. A similar approach to developing environmental quality guidelines could be attempted for species native to the Bay of Fundy and for the three pesticides of interest: deltamethrin, azamethiphos and hydrogen peroxide. Table 10 shows the range of treatment concentrations prescribed for the three formulations used in southwest New Brunswick and the sensitivity of three non-target organisms. Included, for interest, is data on the sensitivity of the target organism, sea lice.

The Atlantic Veterinary College has conducted bioassays with sea lice and Salmosan® or AlphaMax® (Table 10). The response of adult females is somewhat variable with EC_{50} estimates including values below and above recommended treatment concentrations. The data show that, in some cases, sea lice are not sensitive to the active ingredients even at concentrations above the recommended treatment concentration. One statistical outlier was identified in bioassays with AlphaMax® with an EC_{50} of 8.6 µg L^{-1}, 4 times the recommended treatment concentration (Dr. Larry Hammell, Atlantic Veterinary College, personal communication). This variability may be of concern from an efficacy perspective. The concern is magnified by the fact the results are for EC_{50}, i.e., effects for 50% of the population. To expect a reduction in sea lice and a reduction in the necessity to treat sea lice, nearly 100% efficacy must be attained.

The bioassays use indigenous populations of sea lice. They are difficult and expensive to plan and perform. Consequently, the number of assays performed is limited and this may contribute to the variability of responses. These bioassays are vitally important in assessing efficacy trends and a program should be established to routinely conduct bioassays on the target organism (sea lice) to ensure the various therapeutants are effective.

It remains unclear if operational treatments could have impacts on local populations of invertebrates. The risk associated with the use of hydrogen peroxide is low, although very little work has been done with sublethal effects on non-target organisms; certainly none in the southwest NB and the Bay of Fundy. Repeated short term exposure to Salmosan® has been shown to affect survival and reproduction in female American lobsters in a cumulative manner, but the risk has not been assessed.

Table 9. Acute-toxicity rating scales (in ppm) from USFWS (1984).

Relative Toxicity	Aquatic EC or LC_{50} (mg/L)
Super Toxic	< 0.01
Extremely Toxic	0.01–0.1
Highly Toxic	0.1–1
Moderately Toxic	1–10
Slightly Toxic	10–100
Practically Non-toxic	100–1000
Relatively Harmless	> 1000

The chemical properties of hydrogen peroxide (Paramove® 50) and azamethiphos (Salmosan®) indicate they should not be of concern for toxicity via sediment. AlphaMax® has been shown to be extremely toxic in laboratory studies employing waterborne exposures. Deltamethrin, however, should bind to sediment and preliminary studies are underway to determine if sediment-borne AlphaMax® is lethal to benthic invertebrates. Another project underway at St. Andrews Biological Station is employing the use of field-deployed mesocosms to assess the lethality of AlphaMax® and Salmosan®. Preliminary work has also been conducted to explore the effects of these formulations on zooplankton in southwest New Brunswick.

In a previously published State of Knowledge document prepared for DFO, the author has stressed that most of the conclusions regarding risk are based on single-species, lab-based studies (Burridge, 2003). While lab-based studies still represent the best way of comparing toxicities of compounds and the standard methods employed give some confidence in making these comparisons, they lack the complexity of the real world. For example:

- The lipophilic nature of deltamethrin will affect the bioavailability of that compound to non-target organisms.
- Some sensitive life stages may be present for relatively short periods of time that may or may not coincide with sea lice treatments at farm sites (Table 11).
- The duration of exposure is likely to be quite variable in the field depending on tides, winds and currents, for example.
- Some life stages have been shown to be more sensitive than others. The physiological status of organisms can affect response as well. Female lobsters appear to be more sensitive to azamethiphos just before and while they are moulting (Burridge et al., 2005), for example. Incorporating these responses in a comprehensive risk assessment is difficult at best.

The complexity described in the previous statements is magnified by the potential for different formulations to be used at different, yet neighbouring, sites. Each of the three compounds discussed has a different mode of action (Table 10). Sessile or immobile individuals could be exposed to several formulations either in naturally produced mixtures or from sequential

releases. As stated earlier, exposure may be short lived or of long duration. The consequences of this are unknown and extremely difficult to assess or model. Hartwell (2011) and Leight et al. (2005) monitored invertebrate populations in the southeast United States and related the trends in those populations to input of anthropogenic compounds including pesticides. They found lower numbers of Blue crab and Grass shrimp in areas of heavy agricultural runoff. Since bath treatments are applied directly to water, multiple treatments occurring over a time scale of hours to days within and among farms could lead to the potential for non-target organisms being exposed multiple times.

Table 10. LC_{50} (µg L^{-1} of active) of bath treatments used in southwest New Brunswick during 2009-2011. Exposures of lobsters and mysids were conducted at St. Andrews Biological Station and were of 1-h duration followed by 95-h monitoring. The threshold concentrations are measured concentrations of the active ingredient.

Formulation (active ingredient)	Treatment Conc (µg L^{-1} of active)	Mode of Action	Stage I Lobsters	Adult Lobsters	Mysids	Sea Lice*
AlphaMax® (deltamethrin)	2	CNS; chloride channels	0.0034	0.0188	0.0139	0.6-3.0† (n=4)
Salmosan® (azamethiphos)	100	CNS; AChE inhibition	>86.5	24.8	>85.5	15-460 (n=11)
Paramove® 50 (hydrogen peroxide)	1,200,000	Mechanical paralysis	1,500,000	3,750,000	973,000	

**Data courtesy of Dr Larry Hammell, Atlantic Veterinary College. Bioassays were conducted with adult female sea lice and were of 30 minute duration followed by 24-h monitoring.*

† Range of EC_{50}s does not include one statistical outlier (8.6 µg L^{-1}).

Table 11. Location and seasonal distribution of invertebrate species native to southwest New Brunswick that have been tested for their sensitivity to anti-louse bath treatments. The bulk of sea lice treatments take place from May to November.

	Larval lobsters Stages I-III	Juvenile lobsters Stage IV +	Adult lobsters	Mysids	Crangon
Position in water column*	pelagic	subtidal, benthic	subtidal, benthic, epi-benthic	intertidal, subtidal, pelagic or epi-benthic depending on species, habitat and time of year	intertidal, subtidal, epi-benthic
Presence in Bay of Fundy*	June - Sept	July – October or Year-round	Mobile but present year round	Seasonal	Year-round

** Dr Andrew Cooper, DFO, personal communication*

Finally, much of what is known about the biological effects of these three pesticide formulations relates to lethality at varying lengths of exposure. While these data are vital for proper risk assessments, sublethal endpoints, especially those related to reproduction, must be studied. Subtle effects (behavioural, reproductive, etc.) are often not revealed by lab-based acute

exposures, nor are they necessarily captured by short term caging studies *in situ*. For example, Waddy et al. (2002) reported that lobsters treated with an in-feed anti-sea louse therapeutant (emamectin benzoate) molted much earlier than would have been predicted. This serendipitous finding clearly supports the suggestion that pesticides may affect non-targets over longer periods of time and in ways not predicted by looking only at the mode of action. Due to a number of factors, particularly cost and space, these types of studies are rarely conducted or reported.

FUTURE WORK/KNOWLEDGE GAPS

1. Within DFO we are still working, for the most part, on live or die responses. Research should be conducted to determine sub-lethal endpoints especially for sensitive organisms, sensitive life stages, and crucial "activities" such as reproduction and moulting. Behavioural responses of non-target organisms, including avoidance, needs to be studied. Long-term holding and assessment of treated non-targets may be required in order to assess some sublethal endpoints.

2. Work within DFO has largely focused on acute exposure and response. Previous work with Salmosan® showed short-term repeated exposure could result in delayed spawning in female lobsters. Similar studies should be designed to look at latent or delayed responses following single, or multiple, sublethal exposures.

3. DFO research continues to focus mainly on non-target crustaceans. Potential effects on zooplankton need to be considered. Other classes of organisms, at various levels of the food chain, should also be considered in planning research. As a starting point, the effects of bath treatments on organisms commonly found as part of the biofouling community on nets could be assessed.

4. Physico-chemical characteristics dictate the likely fate of chemicals. The K_{ow} of deltamethrin indicates that it is the only bath treatment likely to bind to sediment. Research needs to be planned and conducted to determine the potential effects of sediment bound compounds on benthic invertebrates. Some of this work is being conducted through the Canadian Integrated Multi-trophic Aquaculture Network (CIMTAN) (e.g., polychaete sensitivity) and initial studies have been conducted with Sand shrimp in association with Environment Canada.

5. PMRA registers pesticides based, at least in part, on physical–chemical characteristics of the active ingredient(s). It is clear that the formulation ingredients enhance factors such as solubility, meaning that the physical–chemical data derived using active ingredients may not be appropriate when predicting fate and persistence of the product. In the author's opinion, this is a serious short fall in the registration system.

6. As mentioned previously, potential effects of bath treatments on zooplankton is a concern with organizations representing traditional fisheries. Their concerns have focused on zooplankton loss translating into a loss of food for fish. The question(s) could be expanded to include the possibility that dead plankton contaminated with pesticides could be a food source for other organisms. The effect of pesticide contaminated resources in the food chain is a line of research worth pursuing.

7. While some mesocosm studies are being conducted to compare lab and field responses to the bath treatments, monitoring should be conducted to assess the effects of multiple treatments in small geographic areas and to determine the extent (spatially and temporally) of effects. *In situ* studies are recommended. The presence of dye ensures that exposure has occurred and strengthens interpretation of data. Lab work will be necessary to confirm that dye does not affect toxicity.

8. Research is needed to assess the cumulative effects of multiple exposures to single compounds and/or effects of multiple stressors. Multiple stressors can include exposure to several pesticides, effects of water temperature on responses and effects of water quality on responses, for example.

9. Classes of pesticides are defined by specific modes of action. Organophosphate compounds, such as Salmosan®, act by inhibiting enzyme activity and, as such, its effects can be monitored biochemically. Measuring the effects of deltamethrin and hydrogen peroxide is not as easy. There would be value in determining the extent to which some responses are chemical specific. It is possible, even likely, that the pesticides may elicit a generalised stress response independent of the mode of action of the compound. There are some standard methods for assessing generalised stress. These could be integrated into a suite of endpoint assessments to determine if use of chemicals, in general, affects non-target organisms.

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